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(21) International Application Number: PCT/US92/00205 (22) International Filing Date: 8 January 1992 (08.01.92) (30) Priority data: 638,697 8 January 1991 (08.01.91) US (71) Applicant: IMMULOGIC PHARMACEUTICAL CORPORATION [US/US]; One Kendall Square, Building 600, Cambridge, MA 02139 (US). (72) Inventors: LAMB, Jonathan, R. ; 28 Princes Gardens, Ealing W51SD (GB). O'HEHIR, Robyn, E. ; 57 Dartmouth Road, Willesden Green, London NW2 4EP (GB).		(74) Agents: GRANAHAH, Patricia et al.; Hamilton, Brook, Smith & Reynolds, Two Militia Drive, Lexington, MA 02173 (US). (81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent). Published <i>With international search report.</i>
(54) Title: PEPTIDE-MEDIATED HYPOSENSITIZATION OF THE HUMAN T CELL RESPONSE TO DERMATOPHAGOIDES SPP. (HOUSE DUST MITE) (57) Abstract A method of reducing immune response to an allergen in which a non-allergen derived, non-stimulatory peptide which binds to specific MHC class II molecules of antigen presenting cells is used, as well as non-allergen derived, non-stimulatory peptides useful in the method. <div style="text-align: right; font-size: 2em; font-family: cursive;">BB</div>		

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PEPTIDE-MEDIATED HYPOSENSITIZATION OF THE HUMAN
T CELL RESPONSE TO DERMATOPHAGOIDES
SPP. (HOUSE DUST MITE)

Background of the Invention

- 05 The term "atopic allergy" is applied to a group
of allergies, including asthma, hay fever, allergic
rhinitis, urticaria (hives), eczema, conjunctivitis
and food allergies, all of which are induced by
environmental antigens. The mechanisms include the
10 synthesis of specific immunoglobulin E (IgE) with the
differentiation and growth of effector cells such as
mast cells and eosinophils. The activity of these
effector cells is dependent upon T-helper (CD4+) cells
and their products. In turn, the activation of CD4+
15 T-cells is dependent upon antigen receptors on T-cells
recognizing peptide fragments of foreign proteins
(allergens) in association with self gene products of
the major histocompatibility complex (MHC) expressed
on the surface of antigen presenting cells.
- 20 Allergic IgE-mediated diseases are currently
treated by desensitization procedures that involve the
periodic injection of allergen components or extracts.
Desensitization treatments may induce an IgG response
that competes with IgE for allergen, or they may
25 induce specific suppressor T-cells that block the
synthesis of IgE directed against allergen. These
procedures are not always effective and pose the risk
of provoking an allergic response. A therapeutic
treatment that would decrease or eliminate the
30 allergic-immune response to a particular allergen,
without altering the immune reactivity to other

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foreign antigens or triggering an allergic response itself would be of great benefit to allergic individuals.

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Summary of the Invention

The present invention is a method of reducing T cell response to a selected allergen, in which a non-allergen derived, non-stimulatory peptide is used and interferes with T cell recognition of the allergen, resulting in a decreased T cell response. The present invention is useful in reducing (decreasing or eliminating) hypersensitivity of an individual to an allergen. A non-allergen derived, non-stimulatory peptide is administered to the individual to modulate T cell recognition of the allergen and, as a result, to reduce the individual's immune response to the allergen. In the method of the present invention, the non-allergen derived, non-stimulatory peptide administered is one which binds to selected MHC class II molecules of antigen presenting cells and, as a result, inhibits the specific T-helper cell response. The present invention further relates to peptides and compositions useful in the method of reducing hypersensitivity.

In particular, the present invention relates to a method of reducing hypersensitivity of an individual to *Dermatophagoides* spp. (house dust mite or HDM). It also relates to non-HDM derived non-stimulatory peptides, such as non-stimulating peptide analogs of influenza haemagglutinin which bind to DRw52b class II molecules, useful in the present method.

In another embodiment, the invention relates to an in vitro method of determining the capability of a non-allergen derived, non-stimulatory peptide to reduce the immune response to an allergen. This is achieved by determining whether the peptide binds to specific MHC class II molecules of antigen presenting

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cells and whether binding of the peptide to specific MHC class II molecules modulates the T-cell MHC-restricted recognition of the allergen, thereby reducing the immune response to the allergen.

- 05 Currently used methods of achieving hyposensitization to allergens, including Dermatophagoides spp., depend upon the administration of the components or extracts of allergens. However, these components or extracts are not always successful and can
- 10 themselves provoke allergic responses. The present invention provides an alternative approach to such therapy and is particularly advantageous because desensitization can be achieved while the risk of triggering an allergic response is reduced.

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Brief Description of the Drawings

Figure 1 is a graphic representation of HA S-309 induced inhibition of the polyclonal T-cell response of dust mite allergic individuals to specific
05 allergen.

Figure 2 is a graphic representation of HA S-309 modulation of polyclonal T-cell responses to Dermatophagoides spp., M. tuberculosis and PHA.

Figure 3 is a graphic representation of HA S-309
10 modulation of monoclonal T-cell responses to insolubilized anti-CD3 antibody and Dermatophagoides spp.

Figure 4 is a graphic representation of the binding of HA S-309 to DRw52b HLA class II molecules expressed on the surface of murine fibroblasts.

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Detailed Description of the Invention

The present invention is based on the discovery that a non-allergen derived, non-stimulatory peptide is able to bind to selected MHC class II molecules and inhibit both polyclonal and monoclonal T cell response to an allergen (referred to as an allergen of interest) which causes a T cell response in allergic individuals. As described herein, it has been shown that a non-stimulatory peptide analogue derived from influenza virus hemagglutinin (HA) binds selected MHC class II molecules and inhibits the response of mite-specific CD4+ T cells restricted by the MHC class II molecules. As is also described herein, although polyclonal T cell responses were negatively modulated (decreased) by the peptide, recognition of common recall antigens remained intact. T-cell MHC-restricted recognition is defined as a response of a T-cell resulting from an interaction with a MHC class II molecule of an antigen presenting cell and an allergen derived peptide.

The present invention relates to a method of reducing (decreasing or eliminating) a specific allergic response by an individual to an allergen by administering to the individual a peptide which is not derived from the allergen to which the response occurs, is itself non-stimulatory, and binds to MHC class II molecules which also bind the allergen. In particular, the invention relates to a method of reducing T cell recognition of HDM through the use of a peptide analogue derived from influenza virus HA. As a result, an individual's sensitivity to an allergen such as HDM can be reduced by administering to the individual a therapeutically effective dose of

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a non-allergen derived, non-stimulatory peptide, which binds to specific MHC class II molecules of antigen-presenting cells. This binding inhibits the T-cell response to the allergen and thereby, decreases or
05 eliminates the allergic response.

The non-allergen derived, non-stimulatory peptide used in the present method is one derived from or having essentially the same sequence as a peptide from a source other than the allergen of interest. It can
10 have the same (unmodified) amino acid sequence as occurs in the peptide from which it is derived or can have an altered amino acid sequence (i.e., is a peptide "analogue" which differs from the amino acid sequence as it occurs naturally by deletion, addition
15 or substitution of at least one amino acid). As used herein, the term "derived from" includes both amino acid sequences (peptides) physically obtained from an existing sequence (e.g., by cutting or cleaving using chemical or enzymatic processes) and amino acid
20 sequences which are produced, using known methods such as genetic engineering techniques or synthetic chemistry to have substantially the same sequence as that of an existing sequence. As used herein, the term peptide is intended to include both unmodified and
25 modified amino acid sequences.

The non-allergen derived, non-stimulatory peptide can be any peptide that does not provoke a response to the allergen to which desensitization is desired, binds a site on a MHC class II surface molecule of an
30 antigen-presenting cell to which the allergen can also bind, and inhibits the T-cell response to the allergen. In one embodiment of the invention a peptide

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analogue (designated S-309) derived from the natural sequence of the carboxyl terminus of the HA-1 peptide of influenza haemagglutinin (residues 307-319, with the tryosine at position 309 substituted by serine) is
05 used. An advantage of S-309 is that its binding is restricted to a particular MHC class II molecule, whereas the native peptide is non-restricted in the number of MHC class II molecules it has the capability to bind. As described herein, this peptide analogue
10 has been shown to bind class II molecules which also bind HDM and to inhibit the antigen- dependent response of cloned HDM specific T cells with the same restriction specificity. As described in the Examples, the S-309 peptide analogue, having the sequence:

15 Pro-Lys-Ser-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr,
307 319

has been shown to inhibit polyclonal T cell responses to stimulation induced by D. pteronyssinus and monoclonal T cell responses to stimulation induced by D.
20 pteronyssinus and D. farinae. This suggests that the binding of the peptide to MHC surface molecules prevents activation of the majority of HDM reactive T cells. Possible mechanisms by which the activation of helper T-cells is inhibited include 1) competitive
25 binding of the HA-1 peptide and the T-cell receptor for the MHC surface molecule, and 2) interference with the presentation of processed antigens of the allergen complexed to the MHC surface molecules.

A peptide analogue useful in the present method
30 differs from the normally-occurring peptide by at

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least one amino acid (e.g., an addition, deletion or substitution). Alterations in the amino acid sequence can be made to enhance the ability of the peptide analogue to inhibit T cell response to the allergen.

05 Peptides and peptide analogues can be prepared by a variety of known methods. For example, they can be prepared using Merrifield's procedure of solid-phase peptide synthesis (Merrifield, R.B., J. Am. Chem. Soc., 86: 304 (1964), hereby incorporated by refer-

10 ence). The first step in the solid-phase synthesis of amino acid peptide analogues, as well as other peptides, is the formation of a covalent bond between the C-terminal protected amino acid of the chosen peptide

15 chain is then build up residue by residue by repetitive cycles of deprotection, during which the N-ternamal Boc-protecting (N-tect-bitoxycarboxyl) group is removed by trifluoroacetic acid (TFA). This is followed by neutralization with diisopropylethylamine

20 (DEA) of the amino group left as a salt and coupling of the next amino acid in the sequence. The cycle is repeated until the sequence is completed. After assembly is completed, the peptide is cleaved from the resin and purified. Alternatively, peptides useful in

25 the present invention can be produced using genetic engineering techniques. For example, DNA encoding the desired amino acid sequence can be incorporated into an appropriate expression vector and introduced into a host cell suitable for expression of the encoded

30 product.

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Administration of the non-stimulatory peptide to an individual can be by any route by which a therapeutically effective quantity of the peptide can be delivered. For example, administration can be via a
05 parenteral route, such as by subcutaneous, intravenous or intramuscular administration, transdermal passage or uptake from the respiratory tract. Administration can also be via the gastrointestinal tract, such as by oral or rectal administration. The form in which the
10 peptide is administered (e.g., capsule, tablet, solution, emulsion) will depend, at least in part, on the route by which it is administered. For example, administration by injection would involve the use of physiologic saline or other physiological compatible
15 carrier.

A therapeutically effective amount of a non-stimulatory peptide is that amount which will decrease or eliminate the T-helper cell response to a specific allergen. The therapeutically effective amount will
20 be determined on an individual basis and will be based, at least in part, on consideration of the individual's size, the severity of symptoms to be treated, the result sought, the particular non-stimulatory peptide used, etc. The effective amount
25 can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

The non-allergen derived, non-stimulatory peptides used in the method of the present invention
30 bind to MHC class II molecules and interfere with the recognition of allergen MHC-molecule complexes by T-helper cells. As a consequence, the T-cell response

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is inhibited. This inhibition is reflected by a lack of clonal expansion and the secretion of one or more lymphokines which are soluble factors that have multiple effects on B-cells and other T-cells. Lack
05 of or decrease in a T-cell response to an allergen is referred to as a hyposensitization of the T-cells to the allergen.

Although the present invention is described in terms of non-allergen, non-stimulating peptides of
10 influenza haemagglutinin which is used to modulate the immune response to HDM, it is also possible to identify other non-allergen, non-stimulatory peptides useful for the same purpose or for modulating immune responses to other allergens. Identification of other
15 inhibitors of specific allergens can be achieved by 1) identifying the specificity of the restriction (MHC class II) molecules of importance with the specific allergen; 2) searching the database for peptides known to bind to these molecules; 3) synthesizing and
20 testing the above peptides for their ability to inhibit binding of and/or recognition of the allergen in a competition assay; and 4) modifying peptides to produce peptide analogues with enhanced binding and/or biological activity. Alternatively, peptides can be
25 randomly selected and screened for their capacity to bind specific MHC class II molecules and inhibit allergen-dependent T-cell proliferation.

The invention is further illustrated by the following specific examples, which are not intended to
30 be limiting in any way.

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EXAMPLE 1 Peptide Inhibition of Polyclonal T Cell Responses

Antigens

Lyophilized extracts of *Dermatophagoides farinae* (D. *farinae*) and *Dermatophagoides pteronyssinus* (D. *pteronyssinus*) were the general gifts of Drs. Lowenstein and Schou (ALK, Horsholm, Denmark) and Bencard (Brentford, Middlesex, U.K.), respectively. A soluble extract of *Mycobacterium tuberculosis* (MTSE) was generously provided by Dr. A. Rees (M.R.C. Tuberculosis Unit, Hammersmith Hospital, U.K.). The HA peptide analogue (residues 307-319: serine at 309) and the keratin peptide (residues 1-9) were synthesized using solid phase techniques (Barany, G. and Merrifield, R., Solid phase peptide synthesis In: Gross, E. Meinhofer, J. (Eds), The Peptides, New York, Academic Press (1979) hereby incorporated by reference) on an Applied Biosystems Peptide Synthesizer with Pam resins, t-Boc protected amino acids, and commercially available reagents (Applied Biosystems, Foster City, CA). Peptides were kindly provided by J. Rothbard (ImmuLogic Pharmaceutical Corporation, Palo Alto, CA). The T cell mitogen phytohemagglutinin (PHA-P) was purchased from Sigma Chemicals Co. (St. Louis, MO), and the murine monoclonal anti-CD3 antibody (OKT3) purified from the hybridoma cell line purchased from the ATCC (Rockville, MD).

Preparation of Lymphocytes and Donor
Characterization

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Peripheral blood mononuclear leucocytes (PBMC) obtained from unmedicated atopic adults with symptomatic HDM allergy (perennial rhinitis) were isolated by centrifugation on a discontinuous gradient of Ficoll-Paque (Pharmacia). All subjects had positive skin prick tests to *Dermaphatogoides* spp., and positive HDM radioallergosorbent tests. PBMC were resuspended in complete medium, RPMI-1640 supplemented with A+ or AB+ serum, 2mM L-glutamine and 100 IU/ml of penicillin/streptomycin.

Isolation of Antigen Reactive T cell Clones

HDM specific T cell clones were isolated as described previously (O'Hehir, R.E., et al., Immunobiology, 62: 635 (1987)). Briefly, PBMC (2.5×10^5 /ml) were stimulated with an optimal concentration of *D. farinae* for 7 days in complete medium. Lymphoblasts enriched on Ficoll-Paque were maintained as a long term line in the presence of irradiated (2500 Rads) autologous PBMC, *D. farinae* and interleukin 2 (IL-2, 10% v/v; Lymphocult-T, Biotest Folex, Frankfurt, FRG) and then cloned by limiting dilution from the T cell line. For cloning, viable cells (0.3 cells/well) were plated in Microtest II trays together with irradiated autologous PBMC (5×10^5 /ml), *D. farinae* and IL-2. After 7 days, growing clones were transferred to flat bottom 96-well microtitre trays and, subsequently, to 24 well trays. At each transfer, the clones received filler cells, antigen and IL-2. The clones were maintained and expanded by the addition of IL-2 every 3-4 days, and antigen together with filler cells every 7 days. Prior to

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their use in proliferation assays, the T cell clones were rested for 6-8 days after the last addition of filler cells and antigen. A number of T cell clones specific for *D. Farinae* and others cross-reactive with *D. farinae* and *D. pteronyssinus* were isolated, and the MHC class II restriction specificities of these clones were mapped (O'Hehir, R.E., et al., Immunology, 64: 627 (1988)).

Proliferation Assays

Polyclonal Responses: PBMC (2.5×10^5 /ml) were cultured with soluble antigen in a total volume of 200 μ l of complete medium in 96-well round bottom plates. Peptides were added at various concentrations to selected wells at the initiation of cultures. After 6 days, tritiated methyl thymidine (1μ Ci [3 H]). TdR/well; Amersham International, Amersham, U.K.) was added to the cultures for 8-16 hours and then the cells were harvested onto glass fibre filters. Proliferation as correlated with [3 H] -TdR incorporation was measured at day 7 by liquid scintillation spectroscopy. The results are expressed as mean counts per minute (cpm) for triplicate cultures.

Peptide Inhibition of Polyclonal T Cell Responses

Stimulation induced with *D. pteronyssinus*: Marked proliferation was induced in all cases when PBMC isolated from 5 atopic individuals with symptomatic perennial rhinitis were stimulated with *D. pteronyssinus* at an optimal concentration (range 3-12 μ g/ml). The addition of the HA peptide analogue,

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with the tyrosine at position 309 substituted by serine, to the cultures resulted in marked inhibition of the proliferative response over a concentration range (0.5-100 $\mu\text{g/ml}$). In the presence of the HA peptide, the percentage proliferation as compared to the maximal HDM response achieved varied from 10 to 34%. As illustrated in Figure 1 (striped bar), PBMC stimulated with HDM in the presence of peptide analogue; open bar: PBMC stimulated with HDM in the absence of peptide analogue). In contrast, coculture of the keratin peptide and *D. pteronyssinus* over the same concentration range failed to decrease the observed response as illustrated in Figure 2A. In all cases, PBMC failed to proliferate to either of the peptides alone.

Stimulation induced with *M. tuberculosis* soluble extract: Polyclonal T cell proliferation observed in response to stimulation with MTSE (3 $\mu\text{g/ml}$) was only minimally inhibited by the HA analogue. The maximal inhibitory effect obtained was only 25% (BS) and 40% (MR) at 100 $\mu\text{g/ml}$, with 13% (BS) and 28% (MR) inhibition at 10 $\mu\text{g/ml}$. No inhibition of the anti-mycobacterial T cell response was observed in the presence of the control keratin peptide as illustrated in Figure 2B.

PHA and anti-CD3 induced activation: PHA at a concentration of 1 $\mu\text{g/ml}$ induced marked proliferation in each subject (see Figures 2C i and ii). No inhibitory effect was observed with the addition of either peptide. Similarly, both peptides failed to inhibit the proliferative response of T cells stimulated directly with immobilized anti-CD3 antibody

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and IL-2 that mimics the recognition of peptide/MHC class II complexes as illustrated in Figure 3A i.

EXAMPLE II Peptide Inhibition of Monoclonal T Cell Responses Proliferation Assays

05 Monoclonal Responses: Antigens and antigen reactive T cell clones were obtained as described in Example I. Cloned T cells (5×10^4 /ml) were cultured with soluble antigen in the presence of irradiated autologous PBMC (1.25×10^5 /ml) in a total volume of
10 200 μ l of complete medium in 96-well round bottom plates. Peptides were added to selected wells as described for polyclonal responses. After 60 hours incubation, [3 H] -TdR was added to the cultures for 8-16 hours and then they were harvested as described
15 for the polyclonal T cell proliferation assays.

Cloned T cells (DE9; cross-reactive for both species of *Dermatophagoides* spp., restricted by products of the B1 gene locus DRw12 and degenerate in its restriction specificity for DR2 subtypes and DR8
20 (O'Hehir, R.E., supra (1988), proliferated markedly to *D. pteronyssinus* (3 μ g/ml) presented by autologous irradiated PBMC as accessory cells. The addition of the HA analogue resulted in marked inhibition of the proliferative response in a dose dependent manner,
25 with 60% inhibition at 100 μ g/ml. In contrast, coculture using the keratin peptide failed to inhibit the HDM induced proliferation as illustrated in Figure 3A ii.

D. farinae specific T cell clones (DE12 and DE47)
30 restricted by the DRAB3 gene product DRw52b and T cell

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clone DE9 were stimulated with increasing concentrations of stimulating antigen (*D. farinae*) in the presence of autologous irradiated PBMC as a source of antigen presenting cells. To these cultures the HA analogue was added at a fixed concentration (100 μ g/ml) and T cell proliferation determined. The HA peptide was able to compete with HDM for presentation to the cloned T cells and effectively inhibit the proliferation (see Figures 3B i-iii). However, increasing the concentration of HDM in the assays was associated with a decrease in the inhibition mediated by the HA peptide in each case.

EXAMPLE III Binding of HA Peptide to Antigen

Presenting Cells Expressing DRw52b MHC

Class II Molecules

The cloning of the DR1Dw1, DR2Dw2B5 and DRw52b genes and their co-transfection with the DRA gene into the Ltk fibroblast cell line (DAP3) have been described in Rothbard, J.B., et al., Cell, 52: 515 (1988); Rabourdin-Combe, C. and Mach, B., Nature, 303: 670 (1983); Borsh, J., et al., J. Exp. Med., 162: 105 (1985); Wilkinson, D., et al., J. Exp. Med., 167: 1442 (1988). The transfected fibroblasts were the generous gifts of Drs. R. Leckler, J. Trowsdale and B. Mach.

Murine fibroblasts transfected with the HLA-D region genes, DRw52b, DR1 and DR2Dw2B5 were examined for their capacity to bind the HA analogue as described in Busch, R., et al., Int. Immunol., 2: 442 (1990). After incubation with peptide, containing long chain biotin at the amino terminus, over a

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concentration range, the fibroblasts were then washed and stained with fluorescein avidin D (Vector Laboratories, CA) was added to the cells prior to a further incubation with fluorescein avidin D. To control for
05 differential expression of HLA class II molecules on the fibroblasts, cells were incubated with a fluoresceinated monomorphic anti-HLA-DR antibody (L243, Becton Dickinson, CA) as described in Lampson, L.A. and Levy, R., J. Immunol., 125: 293 (1980). Stained
10 cells were analyzed by flow cytometry using a FASScan analyzer (Becton Dickinson). Only viable cells, identified by their ability to exclude propidium iodide, were analyzed.

To examine the binding of the HA peptide to
15 DRw252b in isolation from other HLA class II molecules, the appropriate genes were transfected in murine fibroblasts. Murine fibroblasts transfected with the DRw52b gene were able to bind the HA peptide in a dose-dependent manner in contrast to the control
20 fibroblasts expressing DR1 or the untransfected cell line (DAP3), as determined by the level of fluorescence as illustrated in Figure 4. Similarly, the HA peptide was able to bind in a dose-dependent manner to murine fibroblasts transfected with DR2Dw2B5 genes,
25 which were functionally able to present HDM to clone DE9.

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CLAIMS

1. A method of reducing T cell response to a selected allergen, comprising contacting T cells with a non-allergen derived, non-stimulatory peptide in the presence of the selected allergen and antigen-presenting cells, under conditions appropriate for interference by the non-allergen derived, non-stimulatory peptide with T cell response to the selected allergen.
05
2. The method of Claim 1 wherein the selected allergen is a house dust mite allergen and the non-allergen derived, non-stimulatory peptide is an influenza virus HA peptide.
10
3. The method of Claim 2 wherein the influenza virus HA peptide has the following sequence:
15
Pro-Lys-Ser-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala--Thr.
4. A method of inhibiting polyclonal T cell response to stimulation by a selected allergen, comprising combining T cells stimulated by the selected allergen with a non-allergen derived, non-stimulatory peptide which interferes with T cell recognition of the selected allergen.
20
5. The method of Claim 4 wherein the selected allergen is a house dust mite allergen and the non-allergen derived, non-stimulatory peptide is an influenza virus HA peptide.
25

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6. The method of Claim 5 wherein the influenza virus HA peptide has the following sequence:
Pro-Lys-Ser-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala--
Thr.
- 05 7. A non-allergen derived, non-stimulatory peptide which inhibits T cell response to a selected allergen.
8. The non-allergen derived, non-stimulatory peptide of Claim 7 which is an influenza virus HA
10 peptide.
9. The non-allergen derived, non-stimulatory peptide of Claim 8 which has the following sequence:
Pro-Lys-Ser-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala--
Thr.
- 15 10. A method of reducing, in an individual, immune response to an allergen, comprising administering to the individual a therapeutically effective amount of a non-allergen derived, non-stimulatory peptide which binds to specific MHC class II
20 molecules of antigen-presenting cells, wherein binding of the non-allergen derived, non-stimulatory peptide to specific MHC class II molecules modulates T cell MHC-restricted recognition of the allergen, thereby reducing the
25 immune response of the individual to the allergen.

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11. A method of reducing, in an individual, immune response to an allergen of house dust mite, comprising administering to the individual a therapeutically effective amount of a non-
05 allergen derived, non-stimulatory peptide which binds to specific MHC class II molecules of antigen-presenting cells, wherein binding of the non-allergen derived, non-stimulatory peptide to specific MHC class II molecules modulates T cell
10 MHC-restricted recognition of the allergen of house dust mite, thereby reducing the immune response of the individual to the house dust mite allergen.
12. The method of Claim 11 wherein the non-allergen, non-stimulatory peptide is an influenza virus HA
15 peptide.
13. The method of Claim 12 wherein the influenza virus HA peptide has the amino acid sequence:
Pro-Lys-Ser-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala--
20 Thr.
14. The method of Claim 11 wherein said MHC class II molecules are products of HLA-DRAB3 class II genes, DRw52 class II molecules.
15. A method of Claim 11 wherein the MHC class II
25 molecules are products of the DRAB1 genes, DRw12 class II molecules.

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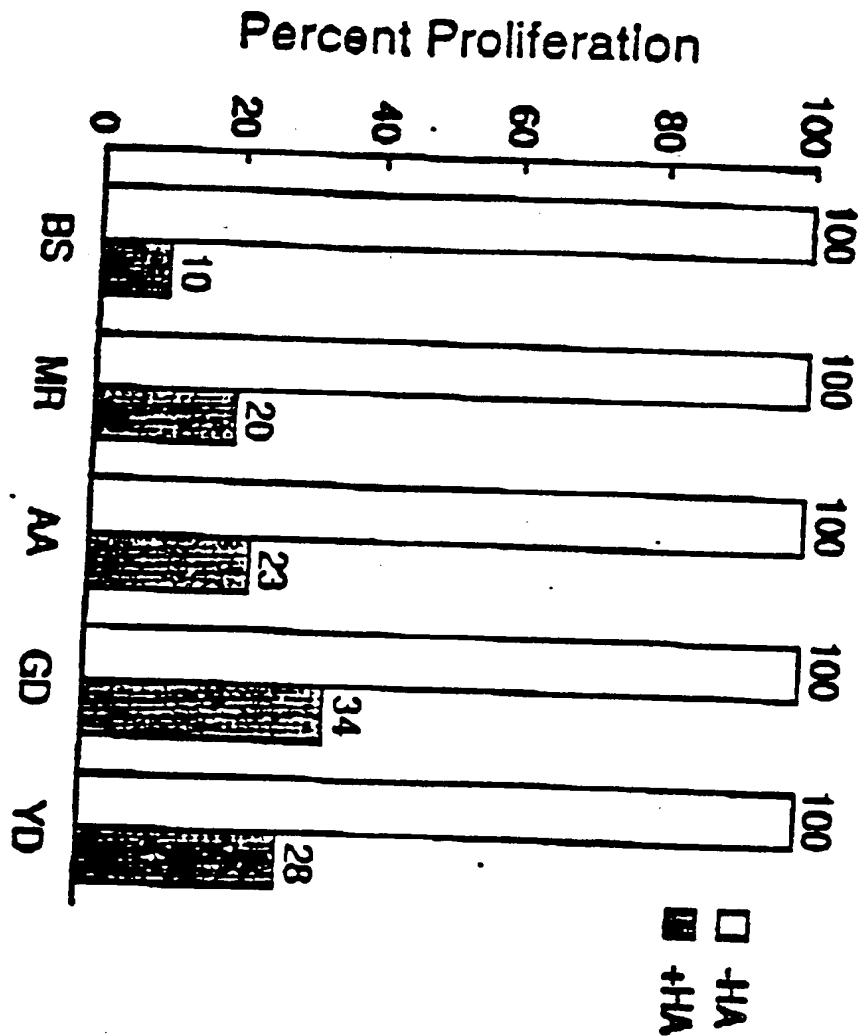
16. A method of reducing, in an individual, immune response to an allergen, comprising administering to the individual a therapeutically effective amount of a non-allergen derived, non-stimulatory peptide of haemagglutinin which binds to DRw52 class II molecules of antigen-presenting cells, wherein binding of the non-allergen derived, non-stimulatory peptide to DRw52 MHC class II molecules modulates T cell DRw52-restricted response to house dust mites, thereby reducing the immune response of the individual to house dust mites.
17. A method of determining the capability of a non-allergen derived, non-stimulatory peptide to reduce immune response to an allergen, comprising determining whether the peptide binds to specific MHC class II molecules of antigen presenting cells and whether binding of the peptide to the specific MHC class II molecules modulates T cell MHC-restricted recognition of the allergen, thereby reducing the immune response to the allergen.
18. A method of determining the capability of a non-house dust mite derived, non-stimulatory peptide to reduce the immune response to a house dust mite allergen, comprising determining whether the peptide binds to specific MHC class II molecules of antigen presenting cells and whether binding of the peptide to the specific MHC class II molecules modulates T cell

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MHC-restricted recognition of house dust mite,
thereby reducing immune response to house dust
mite.

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Figure 1



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Figure 2

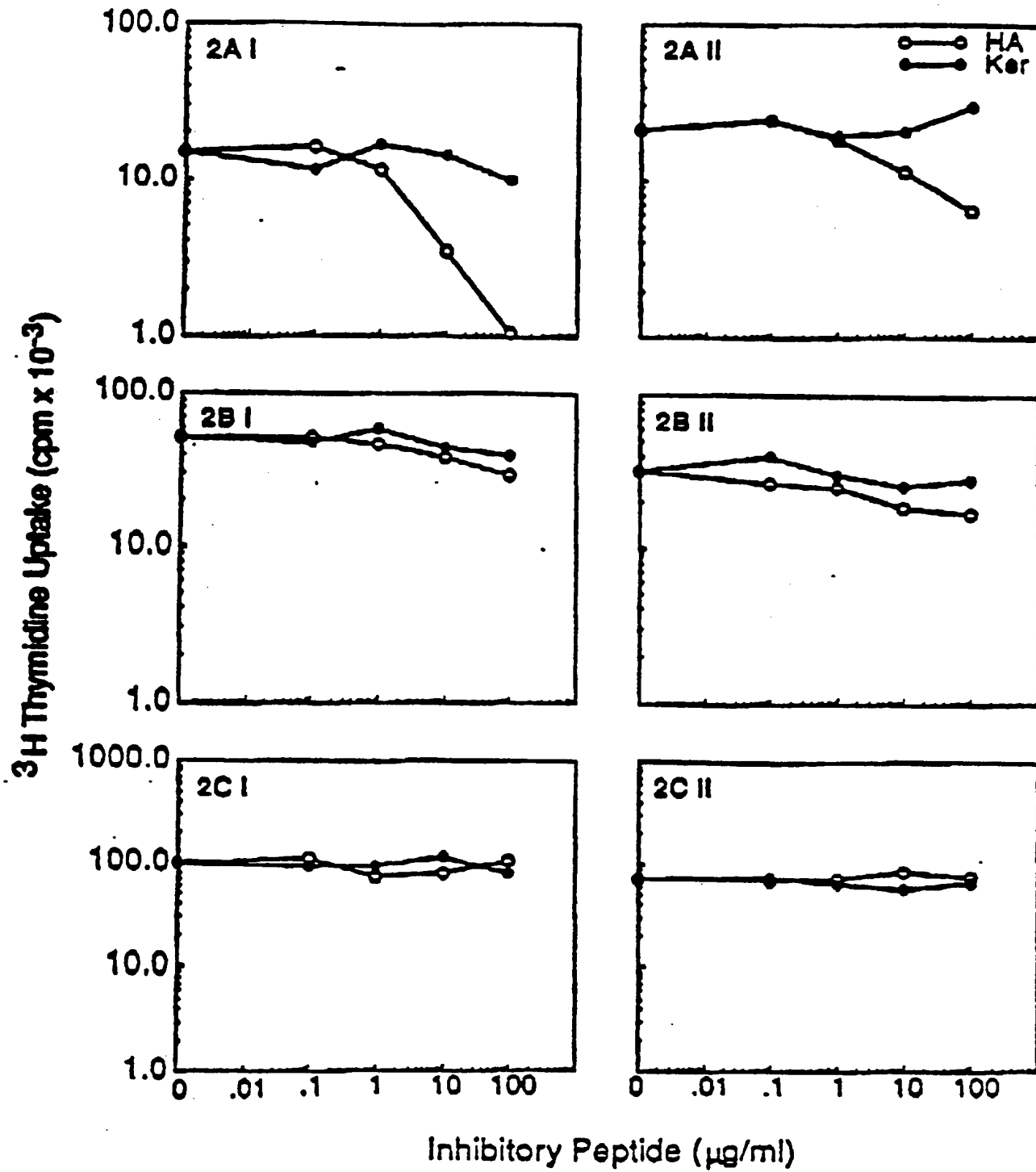
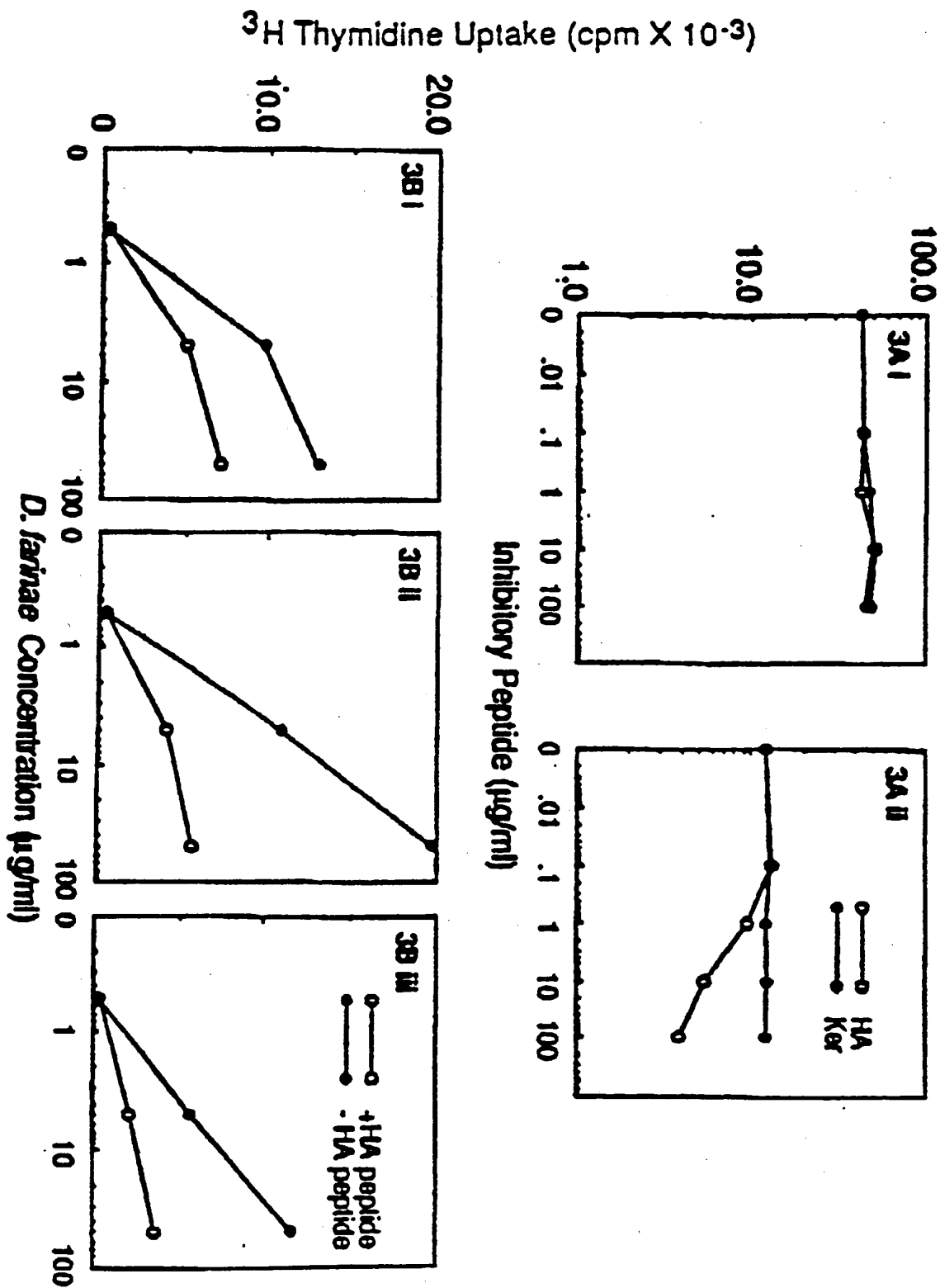
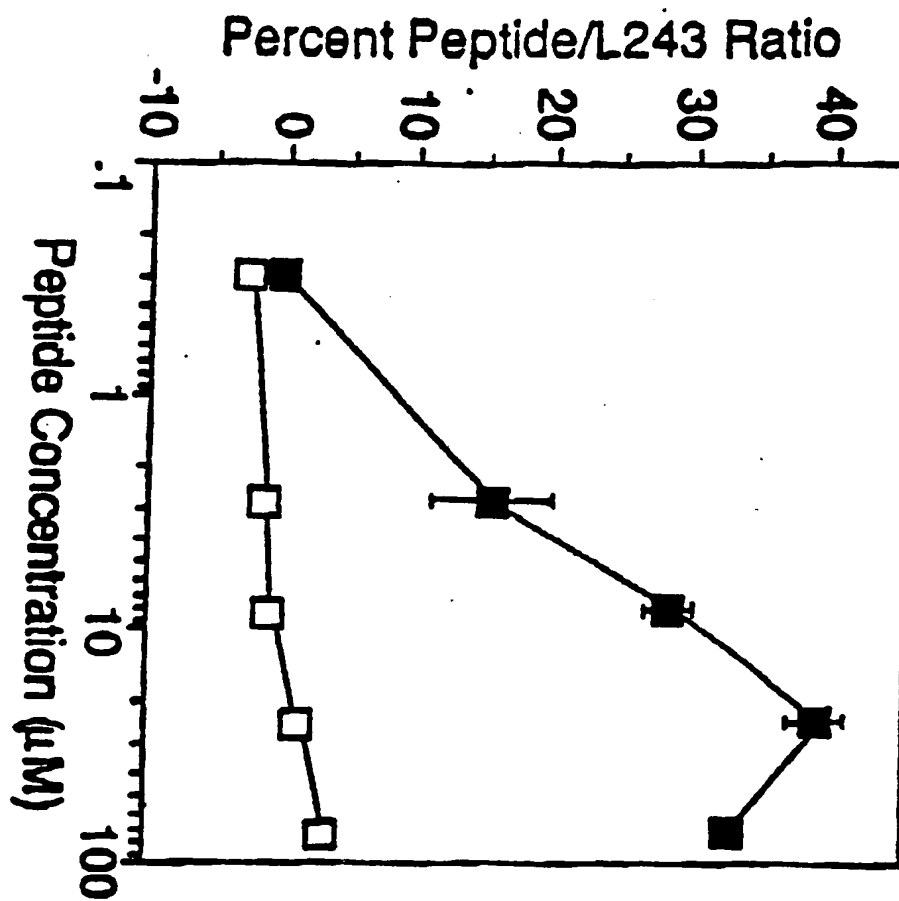


Figure 3



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Figure 4



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/00205

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³ According to International Patent Classification (IPC) or to both National Classification and IPC IPC (5): Please See Attached Sheet. US CL : 514/14; 530/327, 403; 424/7, 85.8																	
II. FIELDS SEARCHED <div style="text-align: center; border: 1px solid black; padding: 2px;">Minimum Documentation Searched⁴</div> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 25%;">Classification System</th> <th style="width: 75%;">Classification Symbols</th> </tr> <tr> <td style="text-align: center; padding: 5px;">U.S.</td> <td style="padding: 5px;">514/14; 530/327, 403; 424/7, 85.8</td> </tr> </table> <div style="text-align: center; border: 1px solid black; padding: 2px;">Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched⁵</div> <p style="padding: 5px;">APS TEXT SEARCH, CAS ONLINE, STN, BIOSIS, MEDLINE</p>			Classification System	Classification Symbols	U.S.	514/14; 530/327, 403; 424/7, 85.8											
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III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴ <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%;">Category*</th> <th style="width: 70%;">Citation of Document,¹⁶ with indication, where appropriate, of the relevant passages¹⁷</th> <th style="width: 20%;">Relevant to Claim No. ¹⁸</th> </tr> <tr> <td style="text-align: center; vertical-align: top;">Y</td> <td style="vertical-align: top;">IMMUNOLOGY, VOL. 64, ISSUED 1988, R. E. O'HEHIR ET AL., "MHC class II restriction specificity of cloned human T lymphocytes reactive with <u>Dermatophagoides farinae</u> (house dust mite)", pages 627-631, see entire document.</td> <td style="text-align: center; vertical-align: top;">1-18</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">Y</td> <td style="vertical-align: top;">NATURE, VOL. 300, issued 04 November 1982, Jonathan R. Lamb et al, "Human T-cell clones recognize chemically synthesized peptides of influenza haemagglutinin", pages 66-68, see entire document.</td> <td style="text-align: center; vertical-align: top;">1-18</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">Y</td> <td style="vertical-align: top;">JOURNAL OF IMMUNOLOGY, VOL. 128, NO. 1, issued JANUARY 1982, JONATHAN R. LAMB ET AL, "ANTIGEN-SPECIFIC HUMAN T LYMPHOCYTE CLONES: INDUCTION, ANTIGEN SPECIFICITY, AND MHC RESTRICTION OF INFLUENZA VIRUS-IMMUNE CLONES", pages 233-238, see abstract.</td> <td style="text-align: center; vertical-align: top;">1-18</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">Y</td> <td style="vertical-align: top;">IMMUNOLOGY, VOL. 62, issued 1987, R.E. O'Hehir et al, "Cloned human T lymphocytes reactive with <u>Dermatophagoides farinae</u> (house dust mite): a comparison of T- and B-cell antigen recognition", pages 635-640, see entire document.</td> <td style="text-align: center; vertical-align: top;">1-18</td> </tr> </table>			Category*	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸	Y	IMMUNOLOGY, VOL. 64, ISSUED 1988, R. E. O'HEHIR ET AL., "MHC class II restriction specificity of cloned human T lymphocytes reactive with <u>Dermatophagoides farinae</u> (house dust mite)", pages 627-631, see entire document.	1-18	Y	NATURE, VOL. 300, issued 04 November 1982, Jonathan R. Lamb et al, "Human T-cell clones recognize chemically synthesized peptides of influenza haemagglutinin", pages 66-68, see entire document.	1-18	Y	JOURNAL OF IMMUNOLOGY, VOL. 128, NO. 1, issued JANUARY 1982, JONATHAN R. LAMB ET AL, "ANTIGEN-SPECIFIC HUMAN T LYMPHOCYTE CLONES: INDUCTION, ANTIGEN SPECIFICITY, AND MHC RESTRICTION OF INFLUENZA VIRUS-IMMUNE CLONES", pages 233-238, see abstract.	1-18	Y	IMMUNOLOGY, VOL. 62, issued 1987, R.E. O'Hehir et al, "Cloned human T lymphocytes reactive with <u>Dermatophagoides farinae</u> (house dust mite): a comparison of T- and B-cell antigen recognition", pages 635-640, see entire document.	1-18
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<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>* Special categories of cited documents:¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> </div> </div>																	
IV. CERTIFICATION <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; padding: 5px;"> Date of the Actual Completion of the International Search² <div style="text-align: center; font-weight: bold;">08 APRIL 1992</div> </td> <td style="width: 50%; padding: 5px;"> Date of Mailing of this International Search Report² <div style="text-align: center; font-weight: bold;">23 APR 1992</div> </td> </tr> <tr> <td style="padding: 5px;"> International Searching Authority¹ <div style="text-align: center; font-weight: bold;">ISA/US</div> </td> <td style="padding: 5px;"> Signature of Authorized Officer²⁰ <div style="text-align: center;"> AVIS DAVENPORT </div> </td> </tr> </table>			Date of the Actual Completion of the International Search ² <div style="text-align: center; font-weight: bold;">08 APRIL 1992</div>	Date of Mailing of this International Search Report ² <div style="text-align: center; font-weight: bold;">23 APR 1992</div>	International Searching Authority ¹ <div style="text-align: center; font-weight: bold;">ISA/US</div>	Signature of Authorized Officer ²⁰ <div style="text-align: center;"> AVIS DAVENPORT </div>											
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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y

IMMUNOLOGY, VOL. 65, issued 1988, P. MAESTRELLI ET AL,
 "ANTIGEN-INDUCED NEUTROPHIL CHEMOTACTIC FACTOR FROM
 CLONED HUMAN T LYMPHOCYTES", PAGES 605-609, see entire
 document.

1-18

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter (1) not required to be searched by this Authority, namely:

2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:

3. ☐ Claim numbers , because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PREVIOUS SHEETS

I. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

A61K 37/00, 37/02, 39/00, 49/00; C07K 3/00, 5/00, 7/00, 15/00, 17/00; G01N 33/15